Very low amounts of glucose cause repression of the stress-responsive gene *HSP12* in *Saccharomyces cerevisiae*

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Changing the growth mode of *Saccharomyces cerevisiae* by adding fermentable amounts of glucose to cells growing on a non-fermentable carbon source leads to rapid repression of general stress-responsive genes like *HSP12*. Remarkably, glucose repression of *HSP12* appeared to occur even at very low glucose concentrations, down to 0.005%. Although these low levels of glucose do not induce fermentative growth, they do act as a growth signal, since upon addition of glucose to a concentration of 0.02%, growth rate increased and ribosomal protein gene transcription was up-regulated. In an attempt to elucidate how this type of glucose signalling may operate, several signalling mutants were examined. Consistent with the low amounts of glucose that elicit *HSP12* repression, neither the main glucose-repression pathway nor cAMP-dependent activation of protein kinase A appeared to play a role in this regulation. Using mutants involved in glucose metabolism, evidence was obtained suggesting that glucose 6-phosphate serves as a signalling molecule. To identify the target for glucose repression on the promoter of the *HSP12* gene, a promoter deletion series was used. The major transcription factors governing (stress-induced) transcriptional activation of *HSP12* are Msn2p and Msn4p, binding to the general stress-responsive promoter elements (STREs). Surprisingly, glucose repression of *HSP12* appeared to be independent of Msn2/4p: *HSP12* transcription in glycerol-grown cells was unaffected in a $\Delta msn2\Delta msn4$ strain. Nevertheless, evidence was obtained that STRE-mediated transcription is the target of repression by low amounts of glucose. These data suggest that an as yet unidentified factor is involved in STRE-mediated transcriptional regulation of *HSP12*.

Keywords: glucose repression, signal transduction, protein kinase A (PKA), glucose 6-phosphate, *HSP12*

INTRODUCTION

Cells of *Saccharomyces cerevisiae* possess the ability to respond rapidly and effectively to changing conditions of the surrounding medium. Indeed, adequate perception of environmental signals and subsequent efficient signal transduction is essential for survival and for proper adjustment to new growth demands. As part of these adaptations to changing growth conditions, the expression level of many yeast genes is modified. When yeast culture conditions become more favourable, tran-

scription of growth-related genes like the ribosomal protein genes is induced, whereas under stress exposure the expression level of the same genes is decreased (Mager & Planta, 1991). Conversely, stress-responsive genes are highly expressed under adverse growth conditions and down-regulated during normal growth (Hohmann, 1997).

We have studied expression levels of the general stress-responsive gene *HSP12* under various growth conditions. This gene, encoding a small heat-shock protein, is expressed under conditions of high osmolarity, heat shock and oxidative stress, during nutrient limitation (Praekelt & Meacock, 1990; Varela *et al.*, 1995) and during growth on non-fermentable carbon sources

Abbreviations: FGM, fermentable growth medium (pathway); PKA, protein kinase A; STRE, stress-responsive element.

(Siderius *et al.*, 1997). The transcriptional regulators Msn2p and Msn4p are involved in the activation of *HSP12* expression during stress conditions (Martinez-Pastor *et al.*, 1996). These factors have been shown to shuttle between cytosol and nucleus in conditions allowing normal growth, and to accumulate in the nucleus in stress conditions and under circumstances of low protein kinase A (PKA) activity (Gorner *et al.*, 1998). Msn2p and Msn4p bind to stress-responsive elements (STREs) that are present in the promoter of many stress-responsive genes (Moskvina *et al.*, 1998) and mediate transcriptional activation of these genes. The promoter of the *HSP12* gene contains five of these elements, which have been shown to be involved in transcriptional regulation under different stress conditions (Varela *et al.*, 1995; Siderius *et al.*, 1997).

We have studied repression of *HSP12* expression under conditions in which the growth potential of the cell increases, by performing carbon-source-shift experiments. Cells growing on a non-fermentable carbon source contain a high level of *HSP12* mRNA (Siderius *et al.*, 1997), which is rapidly diminished when glucose is added to the culture.

Glucose-dependent repression of many genes is regulated via the main glucose-repression pathway. This signalling route is involved in repression of genes involved in the use of alternative carbon sources, and of gluconeogenic genes (Ronne, 1995; Gancedo, 1998). A binding site for the transcription factor Mig1p is present in the promoter of these genes (Nehlin *et al.*, 1991; Lundin *et al.*, 1994). This factor binds to a complex of co-repressors containing Ssn6p and Tup1p, and the total complex confers glucose repression (Treitel & Carlson, 1995).

During the transition from vegetative growth to fermentation, the activation of PKA results in adaptive changes in enzyme activities and gene expression (Thevelein, 1994). PKA is a multisubunit protein kinase consisting of a pair of regulatory subunits encoded by *BCY1* and a pair of catalytic subunits redundantly encoded by *TPK1*, *TPK2* and *TPK3* (Thevelein, 1994). Addition of glucose to a non-fermenting yeast culture results in activation of adenylate cyclase, causing a rapid increase in the cellular level of cAMP. This compound binds to the regulatory subunit of PKA, thereby releasing and activating the catalytic subunits. It is still unclear by what mechanism addition of glucose causes activation of adenylate cyclase. It has been proposed that Ras2p is involved in this process (Jiang *et al.*, 1998). New evidence has shed some doubt on this (Colombo *et al.*, 1998), since it was shown that not Ras2p but rather the G-protein α -subunit Gpa2p and the G-protein-coupled receptor Gpr1p are involved in the increase in cellular cAMP after glucose addition (Yun *et al.*, 1998; Kraakman *et al.*, 1999). Ras2p may be indirectly involved in glucose activation of PKA since it mediates activation of adenylate cyclase upon the intracellular acidification that occurs after addition of sugar to yeast cells (Colombo *et al.*, 1998).

An alternative way of activating PKA, independent of the cellular cAMP level, has been described; this pathway is called the fermentable growth medium (FGM) induced pathway (Thevelein, 1991; Crauwels *et al.*, 1997). Phosphorylation of glucose is not required for activation of PKA via this route, in contrast to activation via cAMP (Pernambuco *et al.*, 1996). The *TPK* homologue protein kinase Sch9p is believed to play a role in this pathway (Crauwels *et al.*, 1997).

In the present study, we used mutants defective in several glucose signalling components to investigate which signal transduction routes play a part in the negative regulation of *HSP12* expression by low amounts of glucose. In addition, we used reporter constructs containing different regions of the *HSP12* promoter to map promoter elements involved in glucose repression.

METHODS

Yeast growth. Yeast cultures were grown on YNB/glycerol medium (0.67% yeast nitrogen base without amino acids, 5% glycerol, necessary amino acids, 0.02% glucose) or YP/ethanol medium (1% yeast extract, 2% peptone, 1% ethanol, necessary amino acids, 0.02% glucose). Genotypes of the strains used in this work are listed in Table 1. *HSP12* promoter–GUS fusions containing 606 (KV3–GUS), 234 (Δ 32–GUS) and 150 (Δ 31–GUS) bp of the 5' flanking region of the *HSP12* gene, respectively, were inserted in the *URA3* locus of W303-1A cells. Single-copy integration was checked by Southern blot analysis.

Carbon-source-shift experiments. Cells were grown to an OD_{660} of 0.2–0.3 (analysed on Beckman DU-6Z spectrophotometer). At zero time, glucose, fructose or 6-deoxyglucose was added in various concentrations. Samples were taken after 10, 30, 60 and 90 min and immediately frozen in liquid nitrogen. Total RNA was isolated as described by Toda *et al.* (1985) and analysed by Northern blot hybridization as described by Varela *et al.* (1995). DNA probes were labelled using the random priming method (Prime-a-Gene labelling system; Promega). Actin signals were used as a loading control and signal quantification was performed by phosphorimaging (Phosphorimager 425; Molecular Dynamics). Relative *HSP12* mRNA levels were calculated by dividing the quantified *HSP12* signal by the *ACT1* signal. The relative *HSP12* signal at time zero was defined as 1, and the *HSP12* levels at other time points are given relative to this starting level.

RESULTS

Carbon-source-dependent regulation of *HSP12* gene expression

Transfer of *S. cerevisiae* from a growth medium with a non-fermentable carbon source to a medium containing a fermentable carbon source leads to major changes in the profile of transcription (Thevelein, 1994). Upon a carbon source upshift, among other changes, ribosomal protein gene transcription – a monitor of the growth potential of yeast cells (Griffioen *et al.*, 1996) – is increased, while transcription of genes under control of the general stress-response pathway is decreased. To study how addition of glucose to yeast cells growing on

Table 1. Genotypes of *S. cerevisiae* strains used

Strain	Genotype	Reference
W303-1A	<i>MATa leu2-4,112 ura3-1 trp1-1 his3-11,15 ade2-1 can1-100 GAL SUC2</i>	Thomas & Rothstein (1989)
$\Delta mig1$	W303-1A <i>mig1::LEU</i>	J. Winderickx, Leuven
MCY 1801	<i>MATα ade2-101 lys2-801 ura3-52 SUC2 ssn6Δ9</i>	Griffioen <i>et al.</i> (1994)
SP1	<i>MATa ade8 his3 leu2 trp1 ura3 can1</i>	Cameron <i>et al.</i> (1988)
RS13-58A-1	SP1 <i>tpk1^{w1}tpk2::HIS3 tpk3::TRP1 bcy1::LEU2</i>	Toda <i>et al.</i> (1985)
$\Delta gpa2$	W303-1A <i>gpa2::URA</i>	Colombo <i>et al.</i> (1998)
$\Delta gpr1$	W303-1A <i>gpr1::LEU</i>	Kraakman <i>et al.</i> (1999)
$\Delta hxx1\Delta hxx2\Delta glk1$	W303-1A <i>hxx1::HIS hxx2::LEU glk1::LEU</i>	De Winde <i>et al.</i> (1996)
$\Delta hxx1\Delta hxx2$	W303-1A <i>hxx1::HIS hxx2::LEU</i>	De Winde <i>et al.</i> (1996)
$\Delta msn2\Delta msn4$	W303-1A <i>msn2::HIS3 msn4::TRP1</i>	Estruch & Carlson (1993)
ENY.WA-1B	<i>MATa leu2 trp1 ura3</i>	Boles <i>et al.</i> (1993)
EBY23	ENY.WA-1B <i>pgi1::URA3</i>	Boles <i>et al.</i> (1993)
YSH312	W303-1a <i>tps1Δ::TRP1 hxx2::LEU2</i>	Hohmann <i>et al.</i> (1993)
Y260	<i>MATa ura3-52 rpb1-1</i>	Nonet <i>et al.</i> (1987)

Fig. 1. Glucose-dependent decrease in *HSP12* mRNA. (a, b) Northern analysis of mRNA levels for *HSP12*, *RPS28A* and *ACT1* after addition of glucose to a final concentration of 2% (a) or 0.02% (b) to cells pre-grown on YNB/glycerol. (c) Growth curve of wild-type cells pre-grown on YNB/glycerol after addition of glucose to a final concentration of 2% (○) or 0.02% (■), or water (▲) as a control. (d) Northern analysis of mRNA levels for *HSP12* and *ACT1* 30 min after addition of glucose in various concentrations to cells pre-grown on YP/ethanol.

a non-fermentable carbon source causes repression of the general stress-responsive gene *HSP12*, we performed carbon-source-shift experiments. Cells were pre-grown on a medium containing glycerol as a carbon source, and in early exponential phase glucose was added to a final concentration of 2%. This experimental condition causes a switch from non-fermentative to fermentative growth (Thevelein, 1994). In a wild-type yeast strain (W303-1A) this leads to a loss of approximately 95% of *HSP12* mRNA within 30 min and a rapid increase in the

expression level of the ribosomal protein gene *RPS28A* (Fig. 1a). Remarkably, however, addition of glucose to a final concentration of only 0.02% also caused a rapid change in *HSP12* and *RPS28A* mRNA levels (Fig. 1b). This low amount of glucose evokes a growth signal (Fig. 1c), although the yeast cells do not switch their metabolism to fermentation. This switch occurs at glucose concentrations above 0.25% (Meijer *et al.*, 1998), which is much higher than the concentrations used in our experiments. The extreme glucose sensitivity

Fig. 2. The main glucose-repression pathway does not mediate glucose-dependent decrease in *HSP12* mRNA. Northern blot analysis of mRNA levels for *HSP12* and *ACT1* after addition of glucose to a final concentration of 2% to cells pre-grown on YNB/glycerol in a $\Delta mig1$ strain (a) and a $\Delta ssn6$ strain (b).

of *HSP12* mRNA levels was confirmed in an experiment in which different concentrations of glucose were used. Final glucose concentrations as low as 0.005% were found to repress *HSP12* expression (Fig. 1d). Experiments using another non-fermentable growth medium (YP/ethanol) revealed a similar glucose repression of *HSP12* (data not shown).

The main glucose-repression pathway is not involved in glucose repression of *HSP12*

Although the low glucose concentration at which *HSP12* repression occurs renders it unlikely that the main glucose-repression pathway plays a part, we investigated the effect of *MIG1* and *SSN6* disruptions on the regulation of *HSP12* expression. Addition of 0.02% glucose to $\Delta mig1$ and $\Delta ssn6$ mutant cells grown on YNB/glycerol medium caused a repression similar to that seen in wild-type cells (data not shown). This result was anticipated since repression via Mig1p has previously been shown not to occur at glucose concentrations lower than 0.2% (Ozcan *et al.*, 1997; Meijer *et al.*, 1998). To assess the possible involvement of the main glucose-repression pathway in *HSP12* gene regulation, the effect of 2% glucose was also examined. As is clear from the data presented in Fig. 2, *HSP12* repression occurred similarly as in wild-type cells, ruling out a role for the main glucose-repression pathway in repression of *HSP12*.

cAMP-dependent activation of PKA is not required for repression of *HSP12*

Addition of glucose to a non-fermenting yeast culture gives rise to a fast increase in the intracellular level of cAMP, which leads to activation of PKA activity (Thevelein, 1994). PKA activity is an important factor in the regulation of *HSP12* expression under stress conditions (Varela *et al.*, 1995; Siderius *et al.*, 1997; Gorner *et al.*, 1998). We therefore examined whether activation of PKA via increase in the cAMP level might also be the mechanism underlying repression of *HSP12* by glucose. However, in agreement with data previously obtained by Beullens *et al.* (1988), also under our culture conditions, addition of glucose to a final concentration of 0.02% did not cause a spike in the cellular cAMP level (data not shown).

To further investigate the putative role of PKA activity in glucose repression of *HSP12*, we made use of the *wimp* mutant ($tpk1^{w1}\Delta tpk2\Delta tpk3\Delta bcy1$), which has a low constitutive level of PKA activity (Cameron *et al.*,

Fig. 3. cAMP/PKA pathway mutants do not affect glucose-dependent decrease in *HSP12* mRNA. (a) Representation of Northern analysis of *HSP12* mRNA levels relative to *ACT1* after addition of glucose to a final concentration of 0.02% to wild-type (■) or $tpk1^{w1}\Delta tpk2\Delta tpk3\Delta bcy1$ (○) cells pre-grown on YNB/glycerol. (b) Representation of Northern analysis of *HSP12* mRNA levels relative to *ACT1* after addition of glucose to a final concentration of 0.02% to W303-1a (■), $\Delta gpr1$ (△) or $\Delta gpa2$ (○) cells pre-grown on YNB/glycerol.

1988). In a 0.02% glucose-shift experiment, *HSP12* levels appeared to drop in this strain as in wild-type cells (Fig. 3a), confirming that cAMP-dependent activation of PKA is not involved in glucose repression of this gene. This result also indicates that direct activation of the catalytic subunits Tpk2p or Tpk3p, which may occur via the FGM pathway (Crauwels *et al.*, 1997), is not essential for glucose repression of *HSP12*.

Recently, the glucose receptor Gpr1p has been identified (Yun *et al.*, 1998; Kraakman *et al.*, 1999); it is coupled to the heterotrimeric G-protein Gpa2p. It has been postulated that Gpa2p rather than Ras2p (Jiang *et al.*, 1998) is implicated in glucose activation of adenylate cyclase and hence PKA activity (Kubler *et al.*, 1997; Lorenz & Heitman, 1997; Colombo *et al.*, 1998; Kraakman *et al.*, 1999). We wished to determine whether the Gpr1p-

Fig. 4. Glucose phosphorylation is essential for glucose-dependent decrease in *HSP12* mRNA. (a) Northern analysis of mRNA levels for *HSP12* and *ACT1* after addition of glucose to a final concentration of 0.02% to $\Delta hxx1 \Delta hxx2 \Delta glk1$ cells pre-grown on YNB/glycerol. (b) Northern analysis of mRNA levels for *HSP12* and *ACT1* after addition of 6-deoxyglucose to a final concentration of 0.02% to W303-1A cells pre-grown on YNB/glycerol. (c, d) Northern analysis of mRNA levels for *HSP12* and *ACT1* after addition of glucose (c) or fructose (d) to a final concentration of 0.02% to $\Delta hxx1 \Delta hxx2$ cells pre-grown on YNB/glycerol. (e) Representation of Northern analysis of *HSP12* mRNA levels relative to *ACT1* after addition of glucose (■) or fructose (□) to a final concentration of 2% to $\Delta pgi1$ cells pre-grown on YNB/ethanol.

Gpa2p system might also be involved in glucose repression as observed for the *HSP12* gene. The data presented in Fig. 3(b) show that this is not the case; *HSP12* repression in both $\Delta gpr1$ and $\Delta gpa2$ cells was the same as for the wild-type. Hence we can also exclude the possibility that an unidentified factor downstream of the glucose receptor Gpr1p plays a part in glucose repression of *HSP12*.

Formation of glucose 6-phosphate is essential for glucose repression of *HSP12*

A key question in understanding the molecular mechanism underlying repression of *HSP12* by low concentrations of glucose is whether glucose acts as a signalling molecule itself or whether it needs to be metabolized to produce the actual signal. Sugar phosphorylation is the first step in glucose metabolism and we found evidence that this step is essential for repression of *HSP12*. We used a yeast strain in which the genes for all three sugar-phosphorylating enzymes are deleted: $\Delta hxx1 \Delta hxx2 \Delta glk1$. Addition of glucose to a final concentration of 0.02% did not cause a drop in *HSP12* mRNA level in this strain (Fig. 4a). Moreover, addition of the glucose analogue 6-deoxyglucose, which can be taken up by the yeast cell but cannot be phosphorylated, did not cause *HSP12* repression in wild-type cells (Fig. 4b). These data strongly suggest that formation of glucose 6-phosphate is required for glucose repression of *HSP12*. However, an alternative interpretation of our findings could be that binding of the substrate glucose to hexokinase may

induce a regulatory function of this protein. Indeed, a regulatory role for Hxk2p has been postulated previously (De Winde *et al.*, 1996; Herrero *et al.*, 1998; Randeziel *et al.*, 1998). Therefore, we aimed to confirm the importance of sugar phosphorylation for signalling, by using a strain in which both *HXX1* and *HXX2* are deleted. The kinase still present, Glk1p, can phosphorylate glucose, but not fructose. Addition of a low amount of glucose to a $\Delta hxx1 \Delta hxx2$ strain caused repression of *HSP12* (Fig. 4c), whereas addition of the same amount of fructose did not (Fig. 4d). These data support our hypothesis that formation of glucose 6-phosphate and not the activity of Hxk2p is essential for *HSP12* repression by low glucose concentrations.

Another way to analyse the importance of glucose 6-phosphate as a potential mediator of regulation is by the use of a $\Delta pgi1$ strain. In this strain the enzyme catalysing the conversion of glucose 6-phosphate into fructose 6-phosphate is absent. Addition of glucose to this strain causes formation of glucose 6-phosphate but no further metabolism can take place (Corominas *et al.*, 1992). Under these conditions *HSP12* was found to be repressed (Fig. 4e). In the control experiment fructose was added. This sugar can be further metabolized, but it cannot be converted into glucose 6-phosphate and, consistent with our model, it does not cause repression.

It could be argued that the effect of the $\Delta pgi1$ mutation on glucose signalling is due to the inability to form trehalose 6-phosphate (from glucose 6-phosphate) rather than the lack of glucose 6-phosphate itself.

Fig. 5. General stress-responsive transcriptional regulators do not affect the glucose-dependent decrease in *HSP12* mRNA. Northern blot analysis of mRNA levels for *HSP12* and *ACT1* after addition of glucose to a final concentration of 0.02% to W303-1A (a) or $\Delta msn2\Delta msn4$ (b) cells pre-grown on YNB/glycerol.

Therefore we examined a mutant with a *TPS1* deletion. In fact, we used a $\Delta tps1\Delta hxx2$ double mutant, since a *Atps1* mutant rapidly dies on glucose unless hexokinase activity is decreased (Hohmann *et al.*, 1993). Addition of glucose to this $\Delta tps1\Delta hxx2$ strain caused repression of *HSP12* to the same level as in the wild-type (data not shown).

Taken together, the results of these experiments led us to conclude that formation of glucose 6-phosphate, but no further metabolism into glycolysis, is essential for the observed repression of *HSP12* by low amounts of glucose. So far it is unknown how this metabolite may mediate signal transmission in yeast.

Msn2p and Msn4p do not play a role in glucose repression of *HSP12*

In order to link the role of glucose 6-phosphate as a mediator of repression to a possible control region in the *HSP12* promoter, we investigated whether known transcriptional regulators binding to this promoter might be involved in this process. Transcriptional activation of general stress-responsive genes is mediated by the transcriptional regulators Msn2p and Msn4p, which bind to STREs (Martinez-Pastor *et al.*, 1996; Schmitt & McEntee, 1996). In the *HSP12* promoter five such STREs are present (Varela *et al.*, 1995). Under conditions of low PKA activity, Msn2/4p are mainly present in the nucleus, in contrast to conditions where PKA activity is high. We assumed therefore that Msn2/4p are present in the nucleus during growth on glycerol and translocate to the cytosol when glucose is added, which might cause the drop in *HSP12* expression. To test this idea, 0.02% glucose was added to a glycerol-grown culture of a $\Delta msn2\Delta msn4$ strain. Surprisingly, this strain displayed normal expression of *HSP12* during growth on glycerol and, moreover, glucose addition caused normal repression of *HSP12* (Fig. 5). These data indicate that Msn2/4p are dispensable for the carbon-source-dependent regulation of *HSP12* expression.

An STRE in the *HSP12* promoter is involved in glucose repression

Since for several yeast genes glucose-dependent regulation of expression has been shown to be under post-transcriptional control (Lombardo *et al.*, 1992; Cereghino & Scheffler, 1996; Scheffler *et al.*, 1998), we investigated whether *HSP12* mRNA stability might be affected by addition of glucose. Initially we attempted to use a strain which carries a temperature-sensitive

Fig. 6. Schematic representation of the *HSP12*-promoter-GUS constructs KV3-GUS, $\Delta 32$ -GUS, $\Delta 36$ -GUS and $\Delta 31$ -GUS. Putative Rap1p- and Abf1p-binding sites are depicted. Heat-shock elements are indicated by HSE, STRE sequences are depicted and a TATA-box is indicated by T.

mutation in the RNA polymerase II subunit Rpb1p (Nonet *et al.*, 1987), which immediately after a shift to the restrictive temperature displays a 95% transcriptional arrest. We measured the rate of decay of *HSP12* mRNA at the restrictive temperature in the presence or absence of glucose, but we could not detect a clear difference. This result may be interpreted as an indication that glucose addition does not cause destabilization of *HSP12* mRNA.

In order to discriminate between the possibilities that glucose addition might affect turnover of *HSP12* mRNA or transcription of the *HSP12* gene, we analysed *HSP12*-promoter-GUS fusion constructs. Fusion of the *HSP12* promoter and the GUS reporter has occurred at the ATG so that all fusion genes contain only the leader sequence of the *HSP12* gene. Thus, if upon glucose addition post-transcriptional regulation occurs, we would expect the same effect on different fusion-gene mRNAs, irrespective of the promoter region present in the respective constructs. We compared the glucose regulation of the fusion gene containing the full promoter (KV3-GUS, see Fig. 6) with $\Delta 31$ -GUS, in which a

Fig. 7. An STRE is involved in glucose repression of the *HSP12* GUS constructs. Northern blot analysis of mRNA levels for *GUS*, *HSP12* and *ACT1* after addition of glucose to a final concentration of 0.02 % to W303-1A cells containing the KV3-GUS (a), $\Delta 31$ -GUS (b), $\Delta 32$ -GUS (c) or $\Delta 36$ -GUS (d) construct pre-grown on YNB/glycerol.

truncated fragment of the *HSP12* promoter, containing no known transcriptional regulatory elements, was fused to a GUS-reporter gene. Expression of the $\Delta 31$ reporter gene appeared not to be subject to glucose repression, whereas the KV3-GUS construct displayed the same regulatory characteristics as the *HSP12* gene itself (Fig. 7a, b). Thus, we can conclude that the drop in *HSP12* mRNA level after addition of glucose is not caused by post-transcriptional regulation, but is accomplished by a transcriptional repression.

In an attempt to identify the promoter element(s) involved in glucose repression, we made use of other GUS-reporter constructs (see Fig. 6). The construct $\Delta 32$ -GUS lacks several known transcriptional-regulator-binding sites present in the full promoter. Nonetheless, when the transformant strain harbouring this construct was subjected to a carbon-source-shift experiment, it showed a substantial decrease in the level of *HSP12* mRNA after 30 min (Fig. 7c). On the other hand, another construct, $\Delta 36$ -GUS, in which the more upstream of the two proximal STREs present in the $\Delta 32$ -GUS construct is deleted, did not show a decrease in GUS-mRNA level 30 min after addition of 0.02 % glucose (Fig. 7d). It is clear from Fig. 7 that KV3-GUS expression differs from that of $\Delta 32$ -GUS at later time points (see Discussion). These results led us to conclude that the STRE located between -234 and -228 is essential for the regulation of the transient glucose repression of the *HSP12* gene.

DISCUSSION

The studies presented in this paper demonstrate that expression of *HSP12* is not only controlled by stress-responsive signalling, but is also regulated by glucose-dependent mechanisms.

Addition of very low amounts of glucose (0.02 %) to non-fermenting yeast cells appeared to cause rapid repression of *HSP12* and induction of *RPS28A* expression. Repression by low concentrations of glucose

has previously also been demonstrated for the gluconeogenic genes *FBP1* and *PCK1* (Yin *et al.*, 1996). Apparently, even a very low amount of glucose is perceived by yeast cells as a growth-triggering signal. We have shown that phosphorylation of the sugar, but no further metabolism into glycolysis, is required for signalling to *HSP12* gene expression. The data supporting this conclusion are that (i) glucose does not cause *HSP12* repression in a $\Delta h x k 1 \Delta h x k 2 \Delta g l k 1$ strain, (ii) fructose does not give rise to *HSP12* repression in a $\Delta h x k 1 \Delta h x k 2$ strain, (iii) 6-deoxyglucose has no repressive effect, and (iv) fructose does not lead to repression in a $\Delta p g i 1$ strain. It is likely therefore that glucose 6-phosphate serves as the signalling molecule, as was shown previously for the gluconeogenic genes *FBP1* and *PCK1* (Yin *et al.*, 1996). However, we cannot exclude the possibility that formation of another non-glycolytic metabolite is involved in repression of *HSP12* (Gonçalves & Planta, 1998).

Previously, partial repression of stress-responsive genes such as *CTT1* and *SSA3* has been shown to occur after addition of 2 % fructose to $\Delta h x k 1 \Delta h x k 2$ cells, suggesting that formation of glucose 6-phosphate is not essential (Pernambuco *et al.*, 1996). We consider it likely that the difference with our data has to do with the final sugar concentration used. Glucose (or fructose) in fermentable amounts may be sensed through the glucose receptor Gpr1p, which activates the adenylate cyclase pathway via Gpa2p (Kraakman *et al.*, 1999). Kraakman *et al.* (1999) observed repression of *HSP12* to be delayed in a $\Delta g p r 1$ or $\Delta g p a 2$ strain. However, we found repression of *HSP12* by low amounts of glucose to be unaffected in $\Delta g p r 1$ or $\Delta g p a 2$ strains. We have not further analysed repression by fermentable amounts of sugar and we are not able to discriminate whether signal transduction under these conditions might be composed of both types of glucose signalling.

The low amounts of glucose causing *HSP12* repression, as well as the results obtained with the $\Delta m i g 1$ and $\Delta s s n 6$

mutants, indicate that the main glucose-repression pathway does not play a role. In addition, evidence was obtained that neither cAMP-dependent activation of PKA, nor cAMP-independent activation of Tpk2p or Tpk3p, underlies the signalling by low amounts of glucose. We cannot exclude the possibility that the activity of Tpk1p (*wimp*) is modulated under these experimental conditions.

Unexpectedly, the transcription factors Msn2p and Msn4p, which play a predominant part in transcriptional activation of *HSP12* under stress conditions (Varela *et al.*, 1995), are not implicated in glucose repression of this gene. This finding fits with the negative results concerning the effect of PKA, since activity of Msn2/4p is largely determined by the cellular PKA activity (Gorner *et al.*, 1998). The level of derepression of *HSP12* in glycerol-grown cells is similar in the *Amsn2Δmsn4* strain as compared to the wild-type. This raises the question, presently under study, how activation (derepression) of *HSP12* in yeast growing on a non-fermentable carbon source occurs.

Given the fact that Msn2/4p are dispensable for repression of *HSP12* by low glucose concentrations, it is even more surprising that an STRE in the promoter of *HSP12* may be the target of this regulation. By making use of *HSP12*-promoter fusion genes the repression phenomenon could be demarcated to a proximal promoter region between -234 and -228, where an STRE is located. Although the rapid decrease in GUS-mRNA after glucose addition occurred similarly for the KV3-GUS and Δ32-GUS constructs, a clear difference could be observed at later time points. This phenomenon needs to be investigated further. We consider it likely that this difference reflects the different promoter regions present in the respective constructs, which may be important for sustained repression or resumption of activation. Note that the KV3-GUS construct contains three distal STREs, which may also play a role in glucose repression of *HSP12*.

Our results suggest that an as yet unidentified factor mediates transcriptional repression of *HSP12* by glucose. We have preliminary evidence, obtained by bandshift analyses, that such an STRE-binding factor, distinct from Msn2/4p, indeed exists. Other workers have reported discrepancies between the effect of STRE deletions and disruption of *MSN2/MSN4* (Ni & LaPorte, 1995; Parrou *et al.*, 1999). It remains to be elucidated if these findings also reflect the action of (a) novel STRE-binding factor(s).

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